Haemophilus somnus: Bovine Reproductive and Respiratory Disease

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Haemophilus somnus causes a variety of clinical problems in cattle including thrombotic meningoencephalitis, septicemia, arthritis, respiratory infections and reproductive failure (1-9). Encephalitic disease has been reliably reproduced (10) and host responses to whole organisms, or extracted antigens have been reported (11,12). Studies of reproductive and respiratory disease have been more limited. In field cases, *H. somnus* has been associated with abortion (2,3,5,6,8) infertility (3,5) and with the normal male or female reproductive tract (12,13,14). Abortion has been reproduced by inoculation of *H. somnus* into the middle uterine artery or by intra-amniotic inoculation but more natural routes have not resulted in abortion or well documented infertility. Although several studies have shown *H. somnus* to be associated with bovine respiratory disease (3,4,5), few have reproduced the disease. Even if all syndromes in the *H. somnus* complex are considered, little has been done on mechanisms of pathogenesis (15,16,17,18), serological diagnosis (11) or subunit vaccines (12a). In order to gain a greater understanding of host parasite relationships in *H. somnus* pneumonia and abortion, and to develop improved methods of diagnosis and control, our research group has developed a multifaceted approach with several overlapping projects ongoing. As described below, we first studied the natural disease, then attempted to reproduce respiratory and reproductive disease in order to study pathogenesis, diagnosis and prophylaxis.

Natural Disease

In order to gain some insight into the relevance of *H. somnus* disease in the Northwestern United States, we reviewed cases submitted to the Washington Animal Disease diagnostic Laboratory (WADDL). From pneumonia cases in 1983 the WADDL bacteriology laboratory reported approximately equal numbers of cases due to *Pasteurella haemolytica, P. multocida* and *H. somnus*. It was not uncommon to isolate two of these three bacteria from the same pneumonic lung. From January 1 to September 30, 1983 the WADDL bacteriology laboratory reported nine cases of *H. somnus* induced abortion where the organism was isolated from the fetal tissues or stomach contents. Mixed infections with other pathogens were not characteristic of abortion in contrast to pneumonia. *Haemophilus somnus* was isolated from several cases of infertility also but it was difficult to interpret the etiological significance of these results, in light of recent reports of *H. somnus* colonization of the genital tract of normal bulls (12,13,14). Consequently we surveyed several herds for the presence of *H. somnus* on mucosal surfaces. This was done to confirm the presence of *H. somnus* in normal bulls as reported by others (12,13), to extend these studies to the female genital tract and to compare reproductive and respiratory colonization. Our data for preputial colonization agreed with data from other studies (12,13) showing that many normal bulls harbor this organism (Table I). Furthermore, our study showed that although females are less often colonized than bulls, the prevalence of 15% positive vaginal cultures indicates that normal heifers and cows often carry *H. somnus* asymptomatically. In both cases, positive nasal cultures were much less common than positive genital cultures.

From these cultural studies of normal herds and observations from clinical cases as well as from published data (1-9,11-13), it can be concluded that *H. somnus* is both a pathogen and a commensal organism. Whether different strains cause disease or result in a commensal relationship is unknown. To answer this question it is necessary to develop experimental disease in the natural host. Since an experimental model had been reported only for encephalitis (10), we needed to develop models for respiratory and reproductive disease.

Experimental Disease

Two experimental models have been developed by our group in order to study pathogenesis, diagnosis and methods of prophylaxis.

TABLE I
H. somnus Isolations from Normal Cattle

Sex	Number Tested	% Positive	
		Nasal	Genital[b]
Male	33	0	61%
Female	244	1.2%	15%

[a]Nasal cultures on 165 animals (2 of 165 positive for *H. somnus*).
[b]Genital (preputial or vaginal).

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Abortion — In our experience, most abortion cases submitted to WADDL appeared to be sporadic rather than herd outbreaks or "abortion storms" and occurred from early to late gestation. This suggested a hematogeneous route of infection, perhaps following respiratory colonization or disease rather than venereal transmission with its characteristic herd outbreaks of infertility and abortion. This reasoning lead us to try to reproduce abortion by intrapulmonary or intravenous inoculation (19). Nineteen heifers or cows at 1.5 to seven months gestation were challenged with 10^{10} *H. somnus* isolate 649 (from an aborted fetus). Eleven of these cattle were inoculated intravenously and eight intrabronchially. In the former group, five abortions occurred and one cow resorbed the next fetus six months later (Table II). Abortions occurred at 3.5 to six months of gestation, usually within the first week after challenge. No abortions occurred after intrabronchial inoculation but one fetus was resorbed within two weeks of challenge.

Haemophilus somnus was isolated from the fetal stomach contents of one fetus and in large numbers from the placenta and/or uterus of all cattle which aborted. No pathological changes were detectable in feti but when placentas were obtained histological lesions of suppurative necrotizing placentitis were consistent with *H. somnus* infection.

TABLE II
H. SOMNUS ABORTION

Route[a]	n	Abortions	Resorptions	Stage	Culture[c]
IV	11	5	1[b]	3-6 mo	3-4+
IB	8	—	1	1.5	?

[a]IV-intravenous, IB-intrabronchial. Inoculated with 10^{10} *H. somnus* 649.
[b]One cow aborted at four months and next fetus resorbed at 35 days.
[c]Fetal stomach, placenta and/or uterus.

Pneumonia — Haemophilus somnus pneumonia is seen in veal calves, dairy replacement calves, feedlot cattle and adult animals. Since calves have been shown to be most susceptible to pneumonia when passive immunity wanes (20), we chose six to eight week old calves for experimental reproduction of disease. Pneumonia was reliably reproduced by intrabronchial inoculation of an isolate recovered from a calf with acute pneumonia (2336). Severity of pneumonia increased as the dose increased for 106 to 10^8 bacteria per animal (21). Grossly, at 24 hours postchallenge there were well demarcated, swollen foci of red to grey to brown consolidation with marked interlobular and subpleural accumulation of fibrin. Acute *H. somnus* pneumonia was characterized histologically by suppurative and necrotizing bronchiolitis and alveolitis, suppurative vasculitis and less frequently, fibrinoid vasculitis (Figure 1 and 2), dilatation and fibrinocellular thrombosis of lymphatics. These findings in experimental disease were generally similar to those reported in naturally occurring disease (22).

Pathogenesis

The fact that *H. somnus* often colonizes the normal bovine genital tract and less commonly the respiratory tract, but also causes severe reproductive or respiratory disease as well as septicemia, is intriguing. This could be due to differences in host defense or bacterial virulence or a combination of these factors. To consider two possible aspects of this phenomenon we decided to investigate interactions between *H. somnus*

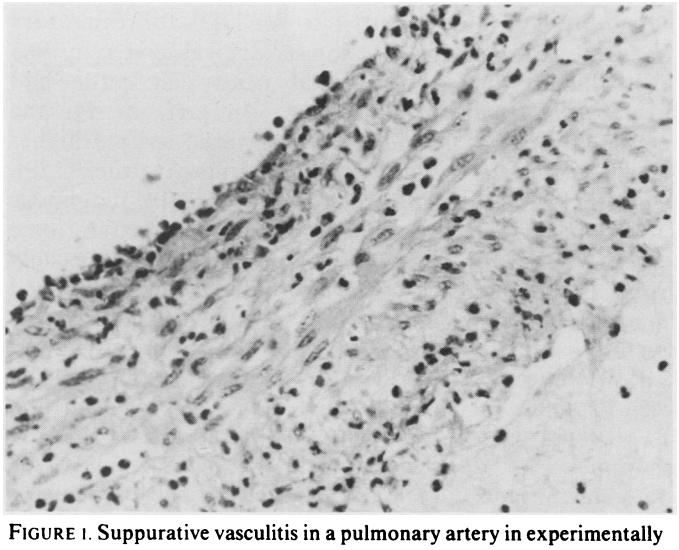

FIGURE 1. Suppurative vasculitis in a pulmonary artery in experimentally produced pneumonia due to *Haemophilus somnus*. There is fragmentation of the tunica media and accumulation of large numbers of neutrophils throughout the vessel wall. H & E. X340.

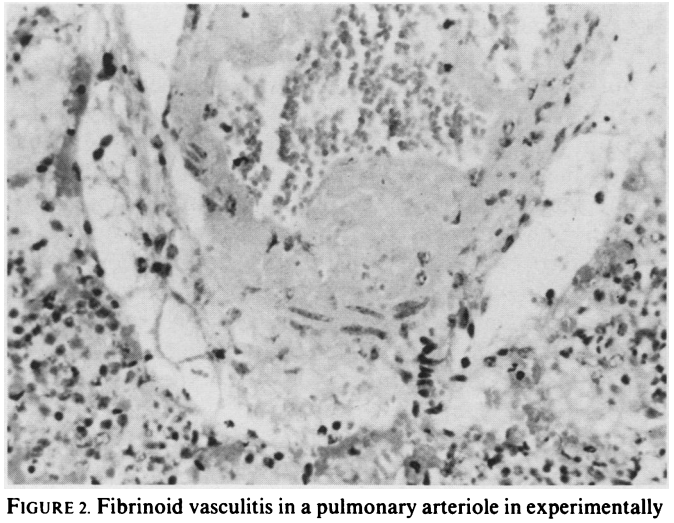

FIGURE 2. Fibrinoid vasculitis in a pulmonary arteriole in experimentally produced pneumonia due to *Haemophilus somnus*. There are large accumulations of fibrinoid material in the vessel wall. Edema of the vascular adventitia and large accumulations of neutrophils, macrophages and erythrocytes are prominent in the alveoli. H & E. X350.

isolates and normal bovine respiratory or genital flora as well as the invasive ability of different *H. somnus* isolates. To study interactions with normal flora, we isolated aerobic bacterial flora from nasal and preputial swabs of normal bulls and determined their ability to enhance or inhibit the growth of six isolates of *H. somnus* in a cross streaking assay (23). Although most normal flora isolates did affect the growth of *H. somnus in vitro*, enhancers outnumbered inhibitors about four to one (Table III). We hypothesize that upsetting the ratio of inhibitors to enhancers could affect the growth of *H. somnus in vivo*.

TABLE III
INFLUENCE OF NORMAL FLORA ON HAEMOPHILUS SOMNUS

Source of Normal flora	Number of normal flora isolates		
	Total	Enhancers[a]	Inhibitors[a]
Nasal	40	35	8
Preputial	30	19	5

[a]Six isolates of *H. somnus* were tested for enhancement or inhbition of growth by each isolate of normal flora.

TABLE IV
SUSCEPTIBILITY OF *H. SOMNUS* ISOLATES TO KILLING BY NORMAL FRESH BOVINE SERUM

Condition	Number of isolates	
	Resistant	Susceptible[a]
Pneumonia	12	1
Reproductive Failure	9	1
Thrombotic Meningoencephalitis	4	0
Preputial Carrier	18	6
Vaginal Carrier	10	1

[a]Greater than 1 log of killing after incubation with normal fresh bovine serum for one hour.

The virulence mechanisms of *H. somnus* have not been well studied but since some isolates have come from invasive disease and others from normal animals, it appeared to us that the ability of isolates to invade may vary. With other Gram-negative bacteria the ability to invade is associated with resistance to killing by fresh normal serum (with functional complement). Consequently we investigated the serum resistance of *H. somnus* isolates from diseased or normal carrier animals (18). Essentially all isolates from clinical disease were resistant to killing by complement in normal serum (Table IV). Two disease isolates were serum susceptible but in each case another pathogen was isolated from the lesion as well. We concluded that the other pathogen was probably the primary etiological agent in these cases. Furthermore, one quarter of the isolates from the normal prepuce were serum susceptible indicating that these isolates may not be invasive. This may account for the lack of reproductive disease in these bulls.

The mechanism of resistance to complement mediated killing is thought to be associated with the composition of the cell surface. Thus far, capsules, lipopolysaccharides and outer membrane proteins (24) have been shown to be responsible for serum resistance in other Gram-negative bacteria. Investigations of these factors for *H. somnus* are underway in our laboratory. Ward and Corbeil (unpublished data) have shown that *H. somnus* has a negatively charged surface similar to the microcapsular antiphagocytic surface of *Campylobacter fetus* (25). However, our attempts to isolate capsular material (T. Inzana, unpublished data) have been negative, lending support to Stephen's (15) conclusion that there is no capsule on *H. somnus*. Inzana (in our laboratory) has ultrapurified lipopolysaccharide (LPS) from three isolates of *H. somnus* and semipurified LPS from an additional dozen isolates. Polyacrylamide gel electrophoresis of LPS preparations followed by periodate oxidation and silver staining, shows that *H. somnus* LPS does not have high molecular weight heterogeneous O side chains like the enteric Gram-negatives. Rather, *H. somnus* LPS appears to be more similar to the lipooligosaccharide of *H. influenzae* (26). Dr. Inzana also has shown that bovine antiserum to whole *H. somnus* organisms reacts with purified LPS of a heterologous strain in enzyme linked immunosorbent assay (ELISA). Dr. Arthur, also in our group, is studying surface proteins of *H. somnus* (27) in parallel with the above studies of LPS. She has found marked differences in protein bands between isolates on SDS-PAGE. Several of these proteins, some of which appear to be associated with the outer membrane, react with convalescent bovine serum in electroimmunoblots, providing evidence that the natural host does produce antibody during infection which could be used diagnostically. Furthermore preliminary studies have shown that the molecular weight of one or two protein bands is different in serum resistant vs. serum susceptible strains suggesting that these proteins may be related to serum resistance.

Diagnosis

Serological methods would be very useful for diagnosis of reproductive and respiratory infection due to *H. somnus*. Some laboratories use a microagglutination test (MAT), which is very sensitive. However, a review of several thousand serum samples submitted to WADDL for respiratory or abortion serological screens in 1983 showed that nearly all cattle had positive titers. In fact, by far the majority were at least tenfold higher than the minimal positive titer (128). To provide a more useful diagnostic test and to determine whether some isotypes of antibody were especially associated with disease, we developed an isotype specific ELISA for *H. somnus* infection (Dowling S, Widders P, Corbeil L — unpublished data). Sera from the experimental abortion study (described above) were tested in this assay and by MAT (Table V). Titers by the MAT were high before inoculation and increased less than twofold. Similarly, IgM titers increased only fourfold by five weeks after inoculation, and IgG1 titers

TABLE V
GEOMETRIC MEAN *H. SOMNUS* TITERS

Time PI[a]	Mat[b]	ELISA IgM	IgG_1	IgG_2
0	1062	56	21	5.1
5 wks	1625	223	250	510

[a]PI — postinoculation
[b]MAT — microagglutination test

increased tenfold, but IgG2 titers increased 100- fold by five weeks and continued to be high at 30 weeks postinoculation (19). This data is consistent with data obtained on clinical samples (Dowling S, Widders P, Corbeil L - unpublished data). These data indicate that an IgG2 specific ELISA may be a useful diagnostic tool for *H. somnus* infection.

Prophylaxis

Although *H. somnus* bacterins are commercially available there is a need for vaccine studies showing efficacy in protecting against experimental pneumonia and abortion. Stephens *et al* (12a) has reported that semipurified anionic outer membrane components are protective against septicemic thrombotic meningoencephalitis. Our preliminary studies showing the convalescent bovine sera recognizes major outer membrane proteins, as well as LPS, gives added evidence that surface components are promising for subunit vaccines.

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